AWARD NUMBER: W81XWH-13-2-0085

TITLE: Understanding Gulf War Illness: An Integrative Modeling Approach

PRINCIPAL INVESTIGATOR: Mariana Morris, PhD

RECIPIENT: Nova Southeastern University
Fort Lauderdale, FL 33314

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2017	Annual	30 Sep 2016 -29 Sep 2017

4. TITLE AND SUBTITLE

Understanding Gulf War Illness: An Integrative Modeling Approach

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-2-0085

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Mariana Morris, PhD; Nancy Klimas, MD; Gordon Broderick, PhD; James PhD; Travis Craddock, PhD and Amanpreet Cheema, PhD

E-Mail: mmorris1@nova.edu, acheema@nova.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Nova Southeastern University, 3301 College Avenue, Fort Lauderdale, FL 33314
Centers for Disease Control, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505
South Florida VA Foundation for Research & Education, Inc., 1201 NW 16th Street, Room #2A103

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of the GWI consortium is to develop a better understanding of GWI and identify specific disease targets to find treatments that will address the cause of the disease. The consortium will integrate our clinical understanding of the disease process with basic research efforts using a novel mathematical model. The computational biology approach will enable the consortium to quickly identify targets of dysfunction and find treatments that will address the causes of the disease. The project will combine animal models of GWI with focus on the immune, cardiovascular and autonomic systems.

15. SUBJECT TERMS
None listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	34	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

.

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The underlying mechanisms of GWI remain unknown and treatment has been palliative, symptom-driven and physician-directed. The purpose of this multidisciplinary consortium project is to investigate animal GWI models with the goal of testing chemical treatments. The immune and autonomic biomarkers will be tested using a computational modeling approach allowing for a critical analysis and an accurate selection of test agents. The idea is to combine animal and human studies – a translational approach. Animal studies will be followed by clinical trials with agents thought to be most efficacious.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Autonomic Dysfunction, Computational Biology, Cytokines, Deregulated Balance, Diisopropyl Phosphorofluoridate , Electrocardiogram, Gulf War Illness, Homeostasis, Molecular Targets, Mouse Model, Putative Therapeutics, Regulatory Network Configuration, Repurposed Drugs, Sarin, Stress Response, Target Intervention, Therapeutic Interventions, Translational Human, Clinical Trials, Translational Model.

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

	Timeline (Months)	**Percentage Complete**
Major Task 1: Setup the administrative structure required for the conduct of the animal and human studies		
Subtask 1: Prepare Regulatory Documents and Research Protocols for Study 1		
Prepare, submit and receive approval for animal protocols	1-4	100%
Refine experimental protocols via conduct of preliminary experiments.	4-12	100%
Refine eligibility criteria, exclusion criteria, screening protocol	3-12	100%
Finalize consent form & human subjects protocol	3-12	100%
Submit amendments, adverse events and protocol deviations as needed	As Needed	100%
Coordinate with Sites for annual IRB** report for continuing review	Annually	0%
Subtask 2: Establishment of administrative structure including coordinating center and database system		
Recruit, hire and train key personnel, students, staff and faculty	1-6	100%
Setup the coordinating center including database setup	1-12	100%
Setup administrative including committee appointments and scheduling of key review meeting	1-5	100%
Development of reporting procedures – minimum of	3-12	100%

updates every 6 months.		
Finalize consent form & human subjects protocol , receive approval	24-36	95%
Annual meeting with the consortium members and the external advisory committee – live and via internet	As scheduled	100%
Meetings in the DC region with DoD staff and representatives of the groups – twice per year	As scheduled	100%

	Timeline (Months)	**Percentage Complete**
Major Task 2: Refinement and enhancement of animal models for GWI.		
Sub task 1: Establish the model of autonomic dysfunction as a surrogate for GWI.		
Train staff and students in specialized surgical methods used to setup for monitoring autonomic function.	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of peripheral autonomic and cardiac function – predict long term deficits	4-15	80%
Employ spectral analytical methods for examination of sympathetic and parasympathetic balance	Begin 4 continue	100%
Conduct wheel running acute and chronic exercise tests to simulate the exercise model in humans	4-12	100%
Combine tests of acute and chronic exercise in the GWI chemical toxin model, providing an excellent preclinical comparison.	12 continue	70%
Submit animal protocol amendments as required	As needed	100%
Measure immune biomarkers in the autonomic dysfunction model, compare to measures of adrenal function	24 continue	35%
Extend preliminary analysis to transcriptional level. Filter and normalize data using accepted best practices and perform traditional analysis of expression profiles at the level of individual genes	6-18 continue	30%
Successful use of data coordination/statistical analysis center bringing together large amounts of data from multiple systems	5 continue	20%
Subtask 2: Establish the model of DFP/CORT as a surrogate for GWI.		
Train staff and students in conduct of model	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of immune markers in brain and periphery	4-15	50%
Employ analytical methods for examination of immunological balance	Begin 4 -16	70%
Establish the minimum levels of corticosterone required to maintained a heightened pro-inflammatory response to the	4-12	100%

sarin surrogate, DFP		
Evaluate stress regimens to establish protocols required to exacerbate proinflammatory response to sarin surrogate, DFP	5-15	70%
Submit animal protocol amendments as required	As needed	100%
Subtask 3: Characterize the molecular and cellular phenotypes of GWI mouse models with the idea of using them to test treatments.		
Use transcriptional analysis to study the immunological basis for the brain and blood changes in the GWI models	12 -24	70%
Use bioinformatic method to estimate pathway activation from gene expression and conduct comparisons between mouse and humans.	6-18	50%
Use molecular modeling to identify and develop networks of expression allowing for robust comparisons between GWI and animal models. Test under baseline and stimulated (stress hormones or exercise)	12-30	70%
Major Task 3: Identification of Illness specific networks with focus on human and mouse comparisons		
Subtask 1: Conduct network analysis for humans and animal models		
Apply biological modeling techniques to pathway activation computed in task 2 sub 3 to render pathway networks	6-12	70%
Integrate with other levels of biology then identify and compare functional modules at various resolutions across groups	12 -18	70%
Conduct detailed analysis of network topology applying measures of network structure and information flow to identify critical information-processing modules	12-24	70%
Conduct an analysis of the alternate steady states available to the regulatory networks identified in human and mouse models.	12-30	70%
Inform pathway-specific genomic panel based on the key network regulatory pathways	12-30	85%
Major Task4: Large-scale simulation of treatment.		
Subtask 1: Conduct in silico sensitivity analysis and rank candidate target nodes		
Use simulation experiments to assess and rank the impact of introducing an in silico equivalent standardized treatment pulse or pulse train at each node in turn throughout the model network	18-30	70%
Rank the candidate target nodes in terms of their relative contribution to shifting the structure of the network recovered under treatment and the network presented in healthy control subjects	18-30	70%

Major Task 5: Define and deploy large-scale optimization.		
Subtask 1: Evaluate and select the best global search algorithm for targeting intervention possibilities		
Review latest developments in evolutionary programming techniques as well as hybrid gradient-based techniques to determine the most suitable search algorithm. Acquire or develop code and deploy.	12-18	50%
Configure simulation-based optimization scheme that evaluates the fitness of candidate interventions by repeatedly launching short network simulation runs in search of the most robust treatment course	18-24	60%
Major Task 6: Identify candidate treatment courses for GWI		
Subtask 1: Using task 5 launch optimization runs from multiple initial conditions of endocrine-immune status		
Identify and describe mathematically the immune and endocrine descriptors that can be effectively and safely changed and over what range they may be changed.	24-30	60%
Using drug databases and bioinformatic techniques identify drugs currently available for repurposing to treat GWI	12-30	60%
Search for novel treatment courses. Launch repeated searches for optimal treatments using the set of candidate cytokine, hormone/autonomic and immune markers isolated in task 5	24-36	15%
Major Task 7: Identify candidate treatment courses for GWI		
Subtask 1: Select and test pharmacological therapies on basis of data from computational models in animals		
Use previous data to select best animal models based on immunological and autonomic biomarkers	24-36	60%
Develop computer/mathematical paradigms for evaluation of treatment strategies	12-30	50%
Develop pilot clinical trials on basis of animal studies	24-36	60%
Major Task 8: Verify treatment effectiveness in human subjects		
Subtask 1: Studies of treatment effectiveness in humans		
Design assessment platform for use in human translational studies using the RedCAP platform as a foundation	18-24	100%
Complete the IRB process for selected study drugs, using the Miami VAMC IRB with OCMR review.	24-30	95%
Recruit and perform assessments of GWI subjects on intervention(s) in the phase 1 translational studies.	30-40	0%
Evaluate change in network interactions from interventions suggested Study 3 and 4. Inform the model with the human study data and refine as necessary	32-48	0%

What was accomplished under these goals?

- Broderick and Craddock attended Gulf War Illness Symposium jointly held with IACFS Annual Meeting in October in Fort Lauderdale, FL. **(Task 1; Subtask2)**
  a) Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelley KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model of Gulf War Illness.
  b) Toole JT, Rice, MA Jr., Cargill J, Craddock TJA, Nierenberg B, Klimas NG, Fletcher MA, Morris M, Zysman J, Broderick G. Increasing Resilience to Traumatic Stress: Understanding the Protective Role of Well-Being.
  c) Craddock TJA, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA, Broderick G. Using gene expression signatures to identify novel treatment strategies in gulf war illness.
  d) Jaundoo R, Bohmann J, Gutierez G, McDonough J, Klimas NG, Broderick G, Morris M, Craddock TJA. Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor for Treatment of Gulf War Illness.
  e) Cournoyer J, Broderick G, Collado F, Fletcher MA, Klimas NG. The Use of the Respiratory Exchange Ratio in Assessing the Metabolic Efficiency of Patients with ME/CFS and GWI.
- Broderick and Craddock participated in the podium talks and participation in panel discussion at GWI Symposium. **(Task 1; Subtask2)**
  f) Broderick G. Complex Illness and Big Knowledge. Symposium on Complex Neuro Inflammatory Conditions: GWI and ME/CFS. Nova Southeastern University, Fort Lauderdale, FL, Oct. 26, 2016.
  g) Craddock TJA. The Puzzle of Life: Using Computational Systems Biomedicine to Make Sense of the Picture. Nova Southeastern University, Fort Lauderdale, FL, Oct. 26, 2016.
  h) Jaundoo R. Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor for Treatment of Gulf War Illness. Nova Southeastern University, Fort Lauderdale, FL, Oct. 26, 2016.
- An abstract was submitted for the Society of Toxicology annual meeting describing GWI animal model in collaboration with CDC. **(Task 1; Subtask2)**
  i) K. A. Kelly, L. T. Michalovicz, J. V. Miller, G. Broderick, M. Rice, T. J. Craddock, M. A. Fletcher, M. Morris, N. Klimas, D. B. Miller, J. P. O'Callaghan. Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. Society of Toxicology 56th Annual Meeting, Baltimore, MD, March 12–16, 2017.
- Dr. Craddock presented, "Harnessing Multi-system Regulation to Identify Optimal Treatment Courses for Complex Chronic Illnesses" (25 min presentation) Translational Medicine, Engineering, and Computing (Trans-MEC) Research Symposium at Nova Southeastern University. Ft. Lauderdale, FL, USA (March 9, 2017). **(Task 1; Subtask2)**
- Craddock TJA, Russell L, Singh SJ, Harvey JM, Rice MA Jr., McKibbin L, Barnes ZM, Nathanson L, O'Callaghan J, Miller DB, Zysman JP, Klimas NG, Fletcher MA, Broderick G. A Logic Model of Neural-Glial Interaction Suggests Altered Homeostatic Regulation in the

Perpetuation of Chronic Neuroinflammation, in-preparation for PLOS Computational Biology. **(Task 1; Subtask2)**

- Jaundoo R, Bohmann J, Gutierez G, Klimas NG, Broderick G, Morris M, Craddock TJA. Using a Consensus Docking Approach to Estimate the Sociability of FDA-Approved Drugs to Predict Repositioning, Polypharmacology and Side-Effects. in-submission with Scientific Reports **(Task 1; Subtask2)**
- Drs. Broderick and Craddock presented detailed review of numerical protocol to Dr. Reifman and other members of EAB at annual review in Fort Detrick on Nov. 30, 2016 **(Task 1; Subtask2).** Highlights include:
  a) Confirmation of feasibility of the proposed numerical protocols involving immune signaling network analysis. Analysis pointed to significant differences in immune signal propagation occur as a result of DFP exposure potentiated by corticosterone.
  b) Reviewed extended comparative analysis of cytokine expression profiles in blood from human GWI veterans with profiles measured in blood of exposed and LPS-challenged mice at 21 days (the basic mouse GWI model). Specifically, evidence for this analysis would suggest that GWI veterans with high Davidson trauma scores (> 70) may constitute a separate immunological sub-type.
- The Broderick group migrated to the Rochester General Hospital, effective February 27, 2017, to establish a new hospital-based Center for Clinical Systems Biology. Dr. Broderick continues to fulfill his role within the Consortium through sub-awards where his new organization has accepted to waive indirect costs. Team members, Saurabh Vashishtha, Mark Rice and Hooman Sedghamiz are now employed at this new Center in Rochester, NY (https://www.rochesterregional.org/CCSB/)**(Task 1; Subtask2).**
- Members of the Consortium submitted and have received acceptance of a manuscript submitted to the journal Brain, Behavior and Immunity as follows:
  a) Ashbrook DG, Hing B, Michalovicz LT, Kelly KA, Miller JV, De Vega W, Miller DB, Broderick G, O'Callaghan J, McGowan PO. Epigenetic impacts of stress priming of the neuroinflammatory response to sarin surrogate in mice: a model of Gulf War Illness. Brain Behav Immun. 2017, In Press. **(Task 1; Subtask2).**
- Morris group presented poster at 12th Biennial International conference of IACFS/ME **(Task 1; Subtask2).**
  a) Machi J, Morris M. Cardiac Function in a Murine Model of Gulf War Illness (GWI) Combination of Organophosphate (DFP) and Exercise Training.
- 2017 SOT 56th Annual Meeting and ToxExpo: Morris presented on cardiovascular and neurotoxin and her team submit posters. **(Task 1; Subtask2).**
  a) Machi J, Morris M. Cardiac Function in a Murine Model of Gulf War Illness (GWI): Success in Therapeutic Trial.
  b) Schmidt R, Morris M. Glucocorticoid Antagonist (Mifepristone) Treatment: Effects on Autonomic Imbalance in a Murine Model of Gulf War Illness.
- Dr. Machi attended the 2017 ESRF 43rd Annual Eastern-Atlantic Student Research Forum (February 22-25, 2017, in Miami FL). She had an oral presentation in a toxicology session, Title: Pre-clinical animal model: Drug treatments are a success to reduce cardiovascular toxicity to DFP exposure. **(Task 1; Subtask2).**
- Dr. Machi attended 2017 European Cardiology Society (ESC) meeting (August 26-30, in Barcelona, Spain) with posters presentation **(Task 1; Subtask2).**:

a) Cardiac Function in a Murine Model of Gulf War Illness: Testing the Model and Phase 1 of Clinical Trial of Etanercept and Mifepristone treatment.
b) Therapeutics drugs and heart disease: A Neurotoxin Model.

- Dr. Machi submitted an abstract to the 2018 SOT 57th Annual Meeting and Tox Expo (March 11-15, in San Antonio, Texas). Entitled: Organophosphate (OP) Toxicity Model in Female Mice - Cardiac Treatment Paradigms. J. F. Machi, R. Schmidt, L. Salgueiro, N. Klimas, and M. Morris. **(Task 1; Subtask2).**
- CDC presented following posters at 56th Annual Meeting of the Society of Toxicology. **(Task 1; Subtask2).**
  a) O'Callaghan J.P., Harry G.J., McPherson C.A., Heijnen C.J., Michalovicz L.T, Klimas N.G. (2017) *Scientific Symposium*: Chemically-induced neuroinflammation and "sickness behavior" disorders: Gulf War Illness. Miller J.V., Kelly K.A., Michalovicz L.T., O'Callaghan J.P., Miller D.B. (2017) Corticosterone-primed neuroinflammatory response to AChE inhibitors is not related to brain acetylcholine concentration.
  b) Kelly K.A., Michalovicz L.T., Miller J.V., Broderick G., Rice M., Craddock T.J., Fletcher M.A., Morris M., Klimas N., Miller D.B., O'Callaghan J.P. (2017) Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model.
- CDC presented poster at 50th Annual Winter Conference for Brain Research. **(Task 1; Subtask2).**
  a) O'Callaghan J.P., Wohleb E.S., Michalovicz L.T., Heijnen C.J., Bowyer J.F. (2017) *Panel Discussion*: Neuroimmune basis of sickness behavior-related disorders.
- CDC has published a manuscript expanding the GWI model to include chlorpyrifos exposure and identifying a lack of direct involvement of AChE inhibition by GW-relevant organophosphates in the neuroinflammation associated with GWI. **(Task 2; Subtask2).**
  a) Locker AR, Michalovicz LT, Kelly KA, Miller JV, Miller DB, and O'Callaghan JP (2017) Corticosterone primes the neuroinflammatory response to Gulf War Illness-relevant organophosphates independently of acetylcholinesterase inhibition *J. Neurochem.* doi: 10.1111/jnc.14071).
- CDC has been accepted to present on GWI as part of the Gulf War Veteran's Illness (GWVI) Symposium at the VA Palo Alto (28-9-2017). **(Task 1; Subtask2).**
- CDC has had 1 poster abstract accepted on data gathered from relevant consortium related projects for the Society for Neuroscience conference in Washington, D.C. (11-11-2017 to 15-11-2017). **(Task 1; Subtask2).**
- O'Callaghan group presented poster at 2017 Expanding Research Partnerships: State of the Science **(Task 1; Subtask2)**. O'Callaghan JP, Kelly KA, Michalovicz LT, Miller JV, Castranova V, Miller DB: Prior exposure to corticosterone markedly enhances and prolongs the response to work-place-related chemical and biological exposures. Abstract & Poster. 2017 Expanding Research Partnerships: State of the Science. Aurora, CO June 2017
- O' Callaghan group had following publications **(Task 1; Subtask2)**:
  a) Kelly KA, Michalovicz L, Miller JV, Miller DB, O'Callaghan JP (2017) Prior exposure to corticosterone markedly enhances and prolongs the neuroinflammatory response to systemic challenge with LPS. PLoS ONE. *Accepted pending revision.*
  b) Koo B-B[§], Michalovicz LT[§], Calderazzo S, Kelly KA, Sullivan K, Killiany RJ, O'Callaghan JP (2017) Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. [§]These authors

contributed equally to the work. Brain Behavior and Immunity. pii: S0889-1591(17)30391-4 doi: 10.1016/j.bbi.2017.08.003.

c) Locker AR, Michalovicz LT, Kelly KA, Miller DB, and O'Callaghan JP (2017) Corticosterone primes the neuroinflammatory response to organophosphate exposure independently of acetylcholinesterase inhibition and without induction of early markers of astrogliosis. J Neurochem. 142(3):444-455.

- Following manuscripts are in preparation **(Task 1; Subtask2)**:
  a) Michalovicz LT[§], Kelly KA[§], Miller JV, Ben-Hamo R, Efroni S, Locker AR, Sullivan K, Broderick G, Miller DB, O'Callaghan JP (2017) The ALDH1L1 bacTRAP mouse as a tool to assess astrocyte specific transcriptome responses to neurotoxicity. [§]These authors contributed equally to the work. *Submitted for Internal CDC-NIOSH Clearance prior to Submission to Glia.*
  b) Miller JV, LeBouf RF, Kelly KA, Michalovicz LT, Miller DB, O'Callaghan JP (2017) Quantification of brain acetylcholine in an acute mouse model of Gulf War Illness. *In Preparation.*
  c) Kelly KA, Michalovicz LT, Miller JV, Locker AR, Miller DB, O'Callaghan JP (2017) Revealing the Gulf War Illness phenotype through systemic inflammatory challenge. *In Preparation.*
  d) Michalovicz LT, Kelly KA, Miller JV, Miller DB, O'Callaghan JP (2017) Microglia play a crucial role in the development and persistence of Gulf War Illness. *In Preparation.*
  e) Kelly KA, Michalovicz LT, Miller JV, Broderick G, Rice M, Craddock T, Fletcher MA, Morris M, Klimas N, Miller DB, O'Callaghan JP (2017) Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. *In preparation.*
  f) Craddock TJA, Kelly KA, Michalovicz LT, Rice Jr. MA, Miller DB, Klimas NG, Morris M, Broderick G, O'Callaghan JP (2017) A logic model of neural-glial interaction suggests altered homeostatic regulation in the perpetuation of chronic neuroinflammation. *In preparation.*
- O'Callaghan group presented following posters at Society for Neuroscience, Washington DC November 2017 **(Task 1; Subtask2):**
  a) Michalovicz LT, Kelly KA, Miller JV, Miller DB, O'Callaghan JP: Microglia play a crucial role in the neuroinflammation underlying Gulf War Illness. Abstract & Poster. Society for Neuroscience, Washington DC November 2017.
  b) Miller JV, Wilmer BM, Kelly KA, Michalovicz LT, Pan CS, O'Callaghan JP, Miller DB: Development of a preclinical rodent model for closed-head non-fatal work-related traumatic brain injury. Abstract & Poster. Society for Neuroscience, Washington DC November 2017.
- O'Callaghan group attended Society of Toxicology, Baltimore, MD March 2017 with following poster presentations **(Task 1; Subtask2):**
  a) Kelly KA, Michalovicz LT, Miller JV, Broderick G, Craddock TJ, Miller DB, O'Callaghan JP: Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. Abstract & Poster. Society of Toxicology, Baltimore, MD March 2017
  b) Miller JV, Kelly KA, Michalovicz LT, O'Callaghan JP, Miller DB: Corticosterone-primed neuroinflammatory response to AChE inhibitors is not related to brain

acetylcholine concentration. Abstract & Poster. Society of Toxicology, Baltimore, MD March 2017.

- Sedghamiz of the Broderick group reviewed preliminary and extended data sets produced by the Morris group **(Task 2; Subtask1).** The latest iteration of this analysis focused on n=11 animals control and n=11 DFP exposed animals without corticosterone priming, the rationale being that the DFP only protocol offers a weaker signal worst case scenario. Despite this weak signal, analysis recovered significant differences ($p<0.05$) in mean and median value in at least 3 of 98 features. These features are aLF (absolute Low Frequency power computed with Lomb-Scargle Periodogram) and parameters describing the fractal structure of the QRS pulse train namely alpha_all and alpha 2 derived using a Detrended Fluctuation Analysis (DFA). These results were reported to the Morris group May 24. This work continues with both teams currently examining corticosterone primed exposures in male and female animals.
- Together with Dr. Vrana and other members of Dr. O'Callaghan's team at CDC/NIOSH, the Broderick group at Rochester General Hospital conducted a series of pathway level simulations in support of a new CDMRP submission. Specifically the team assembled a coarse-grained model of protein regulatory interactions along a subset of core pathways involved in signal transduction propagating downstream of glucocorticoid (NR3C1) and acetylcholine (AChR) receptors. Regulatory associations were extracted using natural language processing of Elsevier's broad scientific literature base. The resulting pathway network consisted of 33 protein and transcription factor nodes, belonging to key signal transduction pathways, connected through 96 causal regulatory interactions (edges). As a first coarse-grain approximation, regulatory dynamics were simulated using simple 2-state (up/down) Boolean logic with transition rules similar to our earlier work under CDMRP award (W81XWH-10-1-0774; Broderick PI). Simulating acute response to DFP exposure, after prior administration of corticosterone, we observed a broad alignment between model predictions (Figure 1) and measurements of 19 proteins expressed in mouse cortex. In addition to those proteins measured, this first model predicts upregulation of protein kinase C α and β and the transcription factor NFATC1 during CORT potentiated response to DFP. We further predict that these remain constitutively upregulated after removal of the stimuli. **(Task 3, Subtask 1).** The objective of the recent submission to CDMRP IIRP is to extend this approach to investigate key signaling phosphoproteins and subsequent pathway perturbations responsible for the neuroinflammatory effects of GW-relevant exposures and target these identified phosphoproteins for therapeutic intervention.
- A dose response for <u>cyclosporine A</u> was performed at CDC and a dose of 10 mg/kg was chosen based on that experiment (Figure 2). This dose will be used to perform the Cyclosporine A/Mifepristone reset experiment (the amendment for this experiment has been approved through the NIOSH ACUC and is awaiting approval from ACURO). **(Task 2, Subtask 2).**
- A dose response for <u>tacrolimus</u> was evaluated at CDC with a dose range of 1-5 mg/kg. The data from this experiment are expected the week of Nov 13. The optimal dose will be chosen and an amendment written to perform the Tacrolimus/Mifepristone reset experiment. **(Task 2, Subtask 2).**
- CDC has completed <u>Enbrel/Mifepristone Reset Therapy</u> experiment (Figure 3). The animals were sacrificed on October 30, 2017. The experiment was done according to the layout included in figure 2 below and results should be in in the next few weeks. **(Task 2, Subtask 2).**

- **Neurohistology**: O'Callaghan group treated mice with CORT (200 mg/L 0.6% EtOH) in the drinking water for 7 days. On the 8th day, DFP (4 mg/kg, i.p.) was administered and animals were given CORT in the drinking water for 7 days in week 3 and 5 at the end of which mice were given LPS (0.5 mg/kg, s.c.) and sacrificed by transcardial formalin perfusion 24 hours later. Brains were removed and sent to FD Neurotechnologies for microglia, astrocyte, and neurodegeneration staining. Previously CDC has shown acute exposure to the initiating event producing GWI pathobiology (CORT and DFP) did not produce significant glial changes in morphology or neurodegeneration (O'Callaghan et al., 2015). Here, we have used that same initiating event with a systemic inflammatory challenge at 5 weeks. While we have slides in hand for these experiments, only a subset of the microscopic images have been taken and thus an incomplete dataset is shown in the figures below. **(Task 3, Subtask 1).**
  Glia: The astrocyte response to the 5 week GWI phenotype is shown in Figure 4. The combination of CORT and DFP was able to create a pathology in which a subsequent, systemic low dose LPS challenge was able to produce astrocyte hypertrophy at 24 hours after exposure. The panels on the right depict representative control and challenged GWI phenotype treated astrocytes at high magnification to highlight the morphological difference in the astrocytes under these conditions. **(Task 3, Subtask 1).**
  Neurodegeneration: Both Silver and Fluro-Jade B neurodegeneration stains were used in this paradigm by CDC labs. Neither Silver (Figure 5) nor Fluoro-Jade B (Figure 6) stains showed significant changes in our model.
- Cardiac function accessed by Morris group using echocardiography showed a decreased ejection fraction in males and females mice after DFP exposure (Figure 7). The drug treatment (Enbrel only, MIF only and Enbrel+MIF) was able to reverse the ejection fraction impairment. The ejection fraction impairment was related to systolic dysfunction, once the echocardiography showed an increased end systolic area in males and females mice after DFP exposure. The end diastolic area accessed by echocardiography did not change significantly after DFP exposure.
  Groups:
  1 – Control Group (Male and Female Intact)
  2 – GWI Model – Cort+DFP (Male and Female OVX)
  3 – Control OVX (Female)
  4 – GWI Model + Enbrel (Male and Female OVX)
  5 – GWI Model + MIF (Male and Female OVX)
  6 – GWI Model + Enbrel + MIF (Male and Female OVX
  Our group is working with Dr. Broderick's group to access the autonomic system by heart rate variability. We are going to meet on November 14th to discuss the results.
- <u>Status for the clinical trial:</u> The IRB at the VA was approved and at NSU was given an expedited review. We have received HRPO approval for the Enbrel Mifepristone clinical trial (letter in appendix). The assessment platform is ready to go, we have the staff trained, and the VA safety officer (the final step at the VA) has authorized us to initiate the clinical trial at their facilities. We had a mock run with everything in place and working smoothly. We will perform a dry run this week before we commence recruitment of participants in the clinical trial. We have a list of prior subjects, and know who met entry criteria, which will be utilized for the recruitment purposes. We hope to have the first subject enrolled before our External Advisory Meeting scheduled Nov 27, 2017.

- **Research grant activity:**

The group was awarded following GWI research awards:

1) GW160101 (Fletcher-Klimas)
   Measurement of Biomarkers in Samples Collected in a Coenzyme Q10 Treatment Trial in Gulf War Illness and Control Subjects.
2) GW160123 (Klimas)
   The Use of B-Cell Depletion Therapy (BCDT) in Gulf War Illness: A Phase 1/2 Study
3) GW160051 (Salgueiro)
   Growth Hormone-Releasing Hormone (GHRH) Antagonist: Evaluation of Beneficial Effects for Gulf War Illness
4) GW160116 (Nathanson)
   Genomics approach to find gender specific mechanisms of GWI pathobiology
5) GW160142 (Morris)
   Gender and Gulf War Illness

The consortium developed two research proposals with the Sullivan group, a continuation of the collaboration of researchers committed to enhance the understanding of the pathobiology of Gulf War Illness and develop targeted treatments for its symptoms:

1) Boston Biorepository, Recruitment, and Integrative Network (BRAIN) for GWI (Sullivan PI, Klimas Co-I; Fletcher Co-I)
2) The Gulf War Illness Clinical Trials and Interventions Consortium (GWICTIC).

Additionally, the group has submitted on several GWIRP applications:

1) Using computational modeling to predict the effect of multiple toxic exposures related to Gulf War Illness pathogenesis to improve diagnostics and treatment (Craddock PI)
2) Immunomodulation in GWI (Nathanson PI; Craddock Co-I)
3) Mild Traumatic Brain Injury Association with Gulf War Illness: Evaluation with Established Models (O'Callaghan PI; Craddock Co-PI)
4) The effect on B cell Subsets during B Cell Depletion Therapy with Rituximab in Patients with Gulf War Illness (Fletcher PI; Klimas Co-I; Craddock Co-I).
5) Neuroinflammation-related phosphoprotein signaling pathways as potential therapeutic targets for GWI using an established animal model (PI: O'Callaghan JP)
6) Mild Traumatic Brain Injury association with Gulf War Illness: Evaluation with established models (PI: O'Callaghan JP)

What opportunities for training and professional development has the project provided?

Faculty, staff, and students are encouraged to present their work at local and national meetings and attend consortium investigator's meetings demonstrated by heavy presence of the group at scientific meetings. There are also continuing opportunities for training.

How were the results disseminated to communities of interest?

The high research activity yielded important findings, which were periodically published, and presented at various scientific meetings.

What do you plan to do during the next reporting period to accomplish the goals?

- Evaluate the effects of Enbrel, Mifepristone or combined treatment in longer-term exposure conditions in a Sarin model (Male protocol).

Member of the **Morris Cardiovascular Toxicology Group** and Dr. **Erik A. Johnson**, Team Leader at the Neuroinflammation and Neuroprotection Research Division, US Army Medical Research Institute of Chemical Defense (USAMRICD) had started to conduct the evaluation of the effect of the combined therapy of Etanercept and Mifepristone in the cardiovascular outcomes resulting after the exposure to Sarin (GB). The study will consider periods of 8 or more weeks post exposure to determine: (1) The long term efficacy of the single administration of these therapeutic agents; and (2) The need of additional therapeutic points to enhance the beneficial effect of these drugs. We have previously demonstrated that the GB administration (0.4 LD50 injected subcutaneously on 2 consecutive days) can cause significant cardiomyopathy (left ventricular dilatation), reduced contractility and autonomic imbalance.

A secondary objective of this approach is to use key biomarkers of cardiovascular pathology (inflammation and remodeling) to evaluate the progress of the cardiovascular outcomes. Based on our recent reports on the effect of this combined therapy to treat acute and persistent signs of GWI (CORT+DFP) in both male and female C57BL/6J mice, we hypothesize that the use of these therapeutic agents can have a long beneficial effect in animals exposed to GB.

Brief SOW description: The designed protocol counts with five basic experimental groups:
Group 1 (Untreated Control no toxic exposure);
Group 2 (Toxic exposed, no treatment);
Group 3 (Single treatment with Enbrel + Toxic exposure);
Group 4 (Single treatment with Mifepristone + Toxic exposure);
Group 5 (Combined treatment with Enbrel and Mifepristone + Toxic exposure).

Important notes: Additional experimental groups can be added to the protocol. New or different therapeutic agents (other than Mifepristone and Enbrel) can be tested in different experimental sets within the study. The flowchart (Figure 8) shows the schedule for the animal exposures, cardiovascular evaluations and experimental endpoints.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Within the last year, critical methods were developed and experiments were completed. This has significant impact on the total project.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
No major changes to report
Actual or anticipated problems or delays and actions or plans to resolve them
The Morris group has experienced some delay related to approval of the animal forms. This should be alleviated within the month.

Changes that had a significant impact on expenditures
Due to delays in approval of animal protocols, the animal experiments are on hold causing delay in expenditures. We just received approval from HRPO for the clinical trial. That has also delayed the expenditures related to the clinical trial. We however are ready to commence the clinical study now with all the necessary approvals and trainings in place, and recruitment will start this week.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
No major changes to report

Significant changes in use or care of vertebrate animals
There are not significant deviations, unexpected outcomes, or changes in approved protocols for the use of vertebrate animals, biohazards, and/or select agents during the reporting period. The following amendments were submitted to the NSU_MM1 and the Miami VA_2856.02 protocols:

NSU_MM1 (GW120045.05):

Amendment/Approval	Approval date	Observation
MM1 protocol	3/23/2017	Protocol Submission
Adding Personnel	4/25/2017	Personnel added: Tracie Rindfield_ animal surgery trainer
Adding personnel	8/1/2017	Non NSU personnel added: Nuno Sacadura; Danielle Talbot; NSU personnel added Alin Benny; Darshan Rola; Matthew Lee; Muthunivas Muthuraj
Adding Substances	8/1/2017	Minor amendment: Use of Lidocaine 2% during surgery to control artery spasm
Surgery procedure	9/21/2017	
Adding personnel	9/17/2017	Personnel added: Marlise DiDomenico

Miami VA_2856.02 (GW120045.02):

Amendment	Approval date	Observation
Protocol 2856.02 renewal	4/20/2017	Three years' renewal.
Adding ovariectomized or normal females to the protocol.	9/7/2016	The PI is requesting to use the same strain C57BL/6J but makes procedure changes. NIH has a new policy to consider female individuals for pre-clinical research protocols intended to inform understanding of diseases and conditions affecting male and females.

Significant changes in use of biohazards and/or select agents
No major changes to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

a) Ashbrook DG, Hing B, Michalovicz LT, Kelly KA, Miller JV, De Vega W, Miller DB, Broderick G, O'Callaghan J, McGowan PO. Epigenetic impacts of stress priming of the neuroinflammatory response to sarin surrogate in mice: a model of Gulf War Illness. Brain Behav Immun. 2017, In Press.
b) Locker AR, Michalovicz LT, Kelly KA, Miller JV, Miller DB, and O'Callaghan JP (2017) Corticosterone primes the neuroinflammatory response to Gulf War Illness-relevant organophosphates independently of acetylcholinesterase inhibition *J. Neurochem.* doi: 10.1111/jnc.14071).
c) Kelly KA, Michalovicz L, Miller JV, Miller DB, O'Callaghan JP (2017) Prior exposure to corticosterone markedly enhances and prolongs the neuroinflammatory response to systemic challenge with LPS. PLoS ONE. Accepted pending revision.
d) Koo B-B§, Michalovicz LT§, Calderazzo S, Kelly KA, Sullivan K, Killiany RJ, O'Callaghan JP (2017) Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. §These authors contributed equally to the work. Brain Behavior and Immunity. pii: S0889-1591(17)30391-4 doi: 10.1016/j.bbi.2017.08.003.
e) Locker AR, Michalovicz LT, Kelly KA, Miller DB, and O'Callaghan JP (2017) Corticosterone primes the neuroinflammatory response to organophosphate exposure independently of acetylcholinesterase inhibition and without induction of early markers of astrogliosis. J Neurochem. 142(3):444-455.
f) Michalovicz LT§, Kelly KA§, Miller JV, Ben-Hamo R, Efroni S, Locker AR, Sullivan K, Broderick G, Miller DB, O'Callaghan JP (2017) The ALDH1L1 bacTRAP mouse as a tool to assess astrocyte specific transcriptome responses to neurotoxicity. §These authors contributed equally to the work. Submitted for Internal CDC-NIOSH Clearance prior to Submission to Glia.

g) Miller JV, LeBouf RF, Kelly KA, Michalovicz LT, Miller DB, O'Callaghan JP (2017) Quantification of brain acetylcholine in an acute mouse model of Gulf War Illness. In Preparation.
h) Kelly KA, Michalovicz LT, Miller JV, Locker AR, Miller DB, O'Callaghan JP (2017) Revealing the Gulf War Illness phenotype through systemic inflammatory challenge. In Preparation.
i) Michalovicz LT, Kelly KA, Miller JV, Miller DB, O'Callaghan JP (2017) Microglia play a crucial role in the development and persistence of Gulf War Illness. In Preparation.
j) Kelly KA, Michalovicz LT, Miller JV, Broderick G, Rice M, Craddock T, Fletcher MA, Morris M, Klimas N, Miller DB, O'Callaghan JP (2017) Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. In preparation.
k) Craddock TJA, Kelly KA, Michalovicz LT, Rice Jr. MA, Miller DB, Klimas NG, Morris M, Broderick G, O'Callaghan JP (2017) A logic model of neural-glial interaction suggests altered homeostatic regulation in the perpetuation of chronic neuroinflammation. In preparation.
l) Craddock TJA, Russell L, Singh SJ, Harvey JM, Rice MA Jr., McKibbin L, Barnes ZM, Nathanson L, O'Callaghan J, Miller DB, Zysman JP, Klimas NG, Fletcher MA, Broderick G. A Logic Model of Neural-Glial Interaction Suggests Altered Homeostatic Regulation in the Perpetuation of Chronic Neuroinflammation, in-preparation for PLOS Computational Biology.
m) Jaundoo R, Bohmann J, Gutierez G, Klimas NG, Broderick G, Morris M, Craddock TJA. Using a Consensus Docking Approach to Estimate the Sociability of FDA-Approved Drugs to Predict Repositioning, Polypharmacology and Side-Effects. in-submission with Scientific Reports.

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers and presentations.

a) Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelley KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model of Gulf War Illness. IACFS-Gulf War Illness Symposium in October in Fort Lauderdale, FL
b) Toole JT, Rice, MA Jr., Cargill J, Craddock TJA, Nierenberg B, Klimas NG, Fletcher MA, Morris M, Zysman J, Broderick G. Increasing Resilience to Traumatic Stress: Understanding the Protective Role of Well-Being. IACFS-Gulf War Illness Symposium in October in Fort Lauderdale, FL
c) Craddock TJA, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA, Broderick G. Using gene expression signatures to identify novel treatment strategies in gulf war illness. IACFS-Gulf War Illness Symposium in October in Fort Lauderdale, FL
d) Jaundoo R, Bohmann J, Gutierez G, McDonough J, Klimas NG, Broderick G, Morris M, Craddock TJA. Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor

for Treatment of Gulf War Illness. IACFS-Gulf War Illness Symposium in October in Fort Lauderdale, FL

e) Cournoyer J, Broderick G, Collado F, Fletcher MA, Klimas NG. The Use of the Respiratory Exchange Ratio in Assessing the Metabolic Efficiency of Patients with ME/CFS and GWI. IACFS-Gulf War Illness Symposium in October in Fort Lauderdale, FL
f) Broderick G. Complex Illness and Big Knowledge. Symposium on Complex Neuro Inflammatory Conditions: GWI and ME/CFS. Nova Southeastern University, Fort Lauderdale, FL, Oct. 26, 2016.
g) Craddock TJA. The Puzzle of Life: Using Computational Systems Biomedicine to Make Sense of the Picture. Nova Southeastern University, Fort Lauderdale, FL, Oct. 26, 2016.
h) Jaundoo R. Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor for Treatment of Gulf War Illness. Nova Southeastern University, Fort Lauderdale, FL, Oct. 26, 2016.
i) K. A. Kelly, L. T. Michalovicz, J. V. Miller, G. Broderick, M. Rice, T. J. Craddock, M. A. Fletcher, M. Morris, N. Klimas, D. B. Miller, J. P. O'Callaghan. Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. Society of Toxicology 56th Annual Meeting, Baltimore, MD, March 12–16, 2017.
j) Machi J, Morris M. Cardiac Function in a Murine Model of Gulf War Illness (GWI) Combination of Organophosphate (DFP) and Exercise Training. 12th Biennial International conference of IACFS/ME.
k) Schmidt R, Morris M. Glucocorticoid Antagonist (Mifepristone) Treatment: Effects on Autonomic Imbalance in a Murine Model of Gulf War Illness. 12th Biennial International conference of IACFS/ME.
l) Machi J, Morris M. Cardiac Function in a Murine Model of Gulf War Illness (GWI): Success in Therapeutic Trial. 2017 European Cardiology Society (ESC) meeting (August 26-30, in Barcelona, Spain)
m) Machi J, Morris M. Cardiac Function in a Murine Model of Gulf War Illness: Testing the Model and Phase 1 of Clinical Trial of Etanercept and Mifepristone treatment. 2017 European Cardiology Society (ESC) meeting (August 26-30, in Barcelona, Spain)
n) Machi M, Morris M. Therapeutics drugs and heart disease: A Neurotoxin Model. 2017 European Cardiology Society (ESC) meeting (August 26-30, in Barcelona, Spain).
o) Machi J, Schmidt R, Salgueiro L, Klimas N, and Morris M. Organophosphate (OP) Toxicity Model in Female Mice - Cardiac Treatment Paradigms. The 2018 SOT 57th Annual Meeting and Tox Expo (March 11-15, in San Antonio, Texas).
p) O'Callaghan J.P., Harry G.J., McPherson C.A., Heijnen C.J., Michalovicz L.T, Klimas N.G. (2017) *Scientific Symposium*: Chemically-induced neuroinflammation and "sickness behavior" disorders: Gulf War Illness.
q) Miller J.V., Kelly K.A., Michalovicz L.T., O'Callaghan J.P., Miller D.B. (2017) Corticosterone-primed neuroinflammatory response to AChE inhibitors is not related to brain acetylcholine concentration. 56th Annual Meeting of the Society of Toxicology
r) Kelly K.A., Michalovicz L.T., Miller J.V., Broderick G., Rice M., Craddock T.J., Fletcher M.A., Morris M., Klimas N., Miller D.B., O'Callaghan J.P. (2017) Resetting

homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. 56th Annual Meeting of the Society of Toxicology.

s) O'Callaghan J.P., Wohleb E.S., Michalovicz L.T., Heijnen C.J., Bowyer J.F. (2017) *Panel Discussion*: Neuroimmune basis of sickness behavior-related disorders. 50th Annual Winter Conference for Brain Research

t) O'Callaghan JP, Kelly KA, Michalovicz LT, Miller JV, Castranova V, Miller DB: Prior exposure to corticosterone markedly enhances and prolongs the response to work-place-related chemical and biological exposures. Abstract & Poster. 2017 Expanding Research Partnerships: State of the Science. Aurora, CO June 2017

u) Michalovicz LT, Kelly KA, Miller JV, Miller DB, O'Callaghan JP: Microglia play a crucial role in the neuroinflammation underlying Gulf War Illness. Abstract & Poster. Society for Neuroscience, Washington DC November 2017.

v) Miller JV, Wilmer BM, Kelly KA, Michalovicz LT, Pan CS, O'Callaghan JP, Miller DB: Development of a preclinical rodent model for closed-head non-fatal work-related traumatic brain injury. Abstract & Poster. Society for Neuroscience, Washington DC November 2017.

w) Kelly KA, Michalovicz LT, Miller JV, Broderick G, Craddock TJ, Miller DB, O'Callaghan JP: Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. Abstract & Poster. Society of Toxicology, Baltimore, MD March 2017

x) Miller JV, Kelly KA, Michalovicz LT, O'Callaghan JP, Miller DB: Corticosterone-primed neuroinflammatory response to AChE inhibitors is not related to brain acetylcholine concentration. Abstract & Poster. Society of Toxicology, Baltimore, MD March 2017.

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Sedghamiz, Broderick and Rice refined and reported as a submission to an upcoming IEEE conference a novel method for efficient feature identification in ECG signals. Sedghamiz H, Rice M, Broderick G. A Real Time Algorithm for Beat Detection and Respiration Estimation in ECG and SCG. IEEE Engineering in Medicine and Biology Society (EMBS), International Conference on Biomedical and Health Informatics (BHI) is "Informatics for precision and preventive medicine". BHI2017, Orlando, FL, Feb. 16-19, 2017. This method is now being applied to Morris group ECG data from GWI animals.

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Mariana Morris, PhD
Project Role:	PI
Research Identifier:	eCommons: mariana
Nearest person month worked:	4.8
Contribution to Project:	Overseeing the entire research project. Established the animal protocols and in charge of the animal research. Oversees hiring all personnel.
Funding Support:	NIH

Name:	Gordon Broderick, PhD
Project Role:	Co-Director
Research Identifier:	eCommons: gbroderick
Nearest person month worked:	1.2
Contribution to Project:	Head of computational biology. Has worked on the computational models for animal and human research to assist i protocols and findings.
Funding Support:	NIH, VA

Name:	Travis Craddock, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: TRAVISCRADDOCK
Nearest person month worked:	2.76
Contribution to Project:	Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, CFIDS Association of America, Nova Southeastern University PFRDG

Name:	Nancy Klimas, MD
Project Role:	Co-Director
Research Identifier:	eCommons: nklimas
Nearest person month worked:	1.2
Contribution to Project:	Head of clinical sciences. Reviewed modeling from the computational biology team in regards to human subjects to hel establish protocols.
Funding Support:	NIH, VA, CDC

Name:	Mary Ann Fletcher, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: mfletche
Nearest person month worked:	1.2
Contribution to Project:	Director of the immunology core.
Funding Support:	NIH, VA

Name:	Amanpreet Cheema, PhD

Project Role:	GWI program administrator
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Monitored budget, maintained meeting schedules, prepared quarterly and annual reports, assisted in establishing sub-awards and other duties associated with administration of the award.
Funding Support:	None

Name:	Jacqueline Machi, PhD
Project Role:	Research Assistant III
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Luis Salguiero, PhD
Project Role:	Laboratory Specialist
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Rodrigo Schmidt
Project Role:	Research Assistant I
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Hooman Sedghamiz
Project Role:	Data Control Specialist
Research Identifier:	None
Nearest person month worked:	5
Contribution to Project:	In charge of the data analysis and has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	No longer funded by this grant

Name:	Saurabh Vashishtha
Project Role:	Research Scientist II: Team Leader/ Network Biology
Research Identifier:	None
Nearest person month worked:	10.2
Contribution to Project:	In charge of the data analysis and responsible for overseeing the design, assembly and validation of prototype network models

	describing undirected and directed interactions between cellular and molecular elements of host and pathogen interaction.
Funding Support:	None

Name:	Jonathan Toole
Project Role:	Research Assistant
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	None

Name:	Labratory Technician
Project Role:	Exercise physiologist
Research Identifier:	None
Nearest person month worked:	2.04
Contribution to Project:	He is responsible for the exercise challenge component.
Funding Support:	None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Klimas has had the following changes to her activity:

Added:

W81XWH-161-1-0632 (Craddock)
DoD GWIRP
Disentangling the Effects of PTSD from GWI for Improved Diagnostics and Treatments
0.144 calendar months

W81XWH-15-1-0163 (PI Waziry)
DoD GWIRP
An Integrated Genomics and Cell Biology Approach to Correlate Novel GWI Indicators of Infections and Neuroinflammatory Mechanisms with Targeted Drug Therapy
0.216 calendar

W81XWH-16-1-0678 (Grant)
DoD GWIRP/ IIREA
Persistently elevated somatic mutation as a biomarker for clinically relevant exposures in GWI
0.36 calendar months

GW160123 (PI Klimas)
DoD GWIRP
The Use of B-Cell Depletion Therapy (BCDT) in Gulf War Illness: A Phase 1/2 Study

0.36 calendar months

GW160051 (PI Salgueiro)
DoD GWIRP
Growth Hormone-Releasing Hormone (GHRH) Antagonist: Evaluation of Beneficial Effects for Gulf War Illness
0.36 calendar months

GW160116 (PI Nathanson)
DoD GWIRP
Genomics approach to find female specific mechanisms of GWI pathobiology
0.216 calendar months

RFA-NS-17-021 (PI Klimas)
NIH
Deconstructing ME/CFS: Using a computational biology framework to identify ME/CFS subtypes and transform treatment
1.2 calendar months

GW160101 (Fletcher-Klimas)
DoD GWIRP
Measurement of Biomarkers in Samples Collected in a Coenzyme Q10 Treatment Trial in Gulf War Illness and Control Subjects
0.36 calendar months
Dr. Fletcher has 5% salary commitment on both these projects. The start of performance period is September, 2017.

Removed:
R56AI120724 (Klimas)
Microbial Discovery and Immunity in ME/CFS
0.36 calendar

Dr. Fletcher has had the following changes to her activity:

Added:
W81XWH-16-1-0678 (Grant)
DoD GWIRP/ IIREA
Persistently elevated somatic mutation as a biomarker for clinically relevant exposures in GWI
0.24 calendar months

GW160142 (Morris)
DoD GWIRP
Gender and Gulf War Illness
0.24 calendar months

GW160051 (PI Salgueiro)
DoD GWIRP
Growth Hormone-Releasing Hormone (GHRH) Antagonist: Evaluation of Beneficial Effects for Gulf War Illness
0.6 calendar months

GW160123 (PI Klimas)
DoD GWIRP
The Use of B-Cell Depletion Therapy (BCDT) in Gulf War Illness: A Phase 1/2 Study
0.6 calendar months

Dr. Morris

Added:
GW160142 (Morris)
DoD GWIRP
Gender and Gulf War Illness
2.16 calendar months

GW160051 (PI Salgueiro)
DoD GWIRP
Growth Hormone-Releasing Hormone (GHRH) Antagonist: Evaluation of Beneficial Effects for Gulf War Illness
0.6 calendar months

W81XWH-15-1-0163 (PI Waziry)
DoD GWIRP
An Integrated Genomics and Cell Biology Approach to Correlate Novel GWI Indicators of Infections and Neuroinflammatory Mechanisms with Targeted Drug Therapy
0.36 calendar months

GW160116 (PI Nathanson)
DoD GWIRP
Genomics approach to find female specific mechanisms of GWI pathobiology
0.24 calendar months

Dr. Salgueiro

Added:
GW160051 (Salgueiro)
DoD GWIRP
Growth Hormone-Releasing Hormone (GHRH) Antagonist: Evaluation of Beneficial Effects for Gulf War Illness.
6 calendar months

Dr. O'Callaghan

Added:
GW160086 (Jones B)
DoD GWIRP
Genetic Basis of Individual Difference in Susceptibility to Gulf War Illness

Dr. Craddock

Added:

GW160116 (PI Nathanson)
DoD GWIRP
Genomics approach to find female specific mechanisms of GWI pathobiology
0.48 calendar

GW150199 (Craddock)
DoD GWIRP
Improving Diagnostics and Treatments for GWI Females by Accounting for the Effects of PTSD
2.4 calendar months

GW150144 (Craddock)
DoD GWIRP
Disentangling the Effects of PTSD from GWI for Improved Diagnostics and Treatments.
2.4 calendar months

Dr. Broderick has 10% salary commitment on GW150199, and GW150144.

What other organizations were involved as partners?

Name:	Centers for Disease Control and Prevention National Institute for Occupational Safety and Health
Location:	1095 Willowdale Road Morgantown, WV 26505
Contribution:	Chemical toxicology project collaboration
Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None
Name:	Southwest Research Institute
Location:	5220 Culebra Road, PO Drawer 28510 San Antonio, TX 78228
Contribution:	Assisting on drug choices to test in animals and humans.
Financial:	None

In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None
Name:	South Florida VA Foundation for Research & Education Inc.
Location:	1201 NW 16th Street, Room #2A103 Miami, FL 33125
Contribution:	Providing subjects and space for human trials in future. Help with establishing human protocols.
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner's facilities for project activities.
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other's facilities. Dr. Klimas and Dr. Fletcher are on staff at Nova Southeastern University and the Miami VA.
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc. – Animal Facility
Location:	1201 NW 16th Street, Room #2A102 Miami, FL 33125
Contribution:	
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner's facilities for project activities.
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other's facilities. Drs. Morris and Salgueiro has been fully moved to Nova Southeastern University but maintains a witho compensation position at the Miami VA.
Other:	None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Nothing to Report

QUAD CHARTS: See next page.

Understanding Gulf War Illness: An Integrative Modeling Approach

Award Number: GW120045 / W81XWH-13-2-0085

PI: Dr. Mariana Morris **Org:** Nova Southeastern University **Award Amount:** $4,102,527

Approach To develop a translational model of GWI for rapid identification of molecular targets and prediction of effective therapeutic interventions. The effectiveness of candidate treatment in terms of system abatement and recovery of regulatory network configuration will be assessed in GWI subjects in phase 1 translational studies.

- ***Study 1:*** Characterize the autonomic neural/adrenal dysfunction in a mouse model of GWI using validation and direction from computational biology (**Task 2**).
- ***Study 2:*** Characterize the molecular and cellular phenotype of GWI in a mouse model to evaluate the role of stress response in persistence of the illness (**Task 2**).
- ***Study 3:*** Integrate human (previously completed) and animal studies using computational biology to identify mediators of deregulated balance and test putative therapeutics (**Task 3-5**)
- ***Study 4:*** Evaluate therapeutics suggested by computational model in GWI animal models. Two or three most favorable will move on to human testing (**Task 6-7**).
- ***Study 5:*** Perform translational human clinical trials to evaluate homeostasis "reset" as well as preliminary safety and efficacy (**Task 8**).

Accomplishments to date

1-The consortium presented GWI research findings at Society of Toxicology conference, Society for Neuroscience, Trans-MEC) Research Symposium, 2017 ESRF 43rd Annual Eastern-Atlantic Student Research Forum, 2017 European Cardiology Socie and 50th Annual Winter Conference for Brain Research .

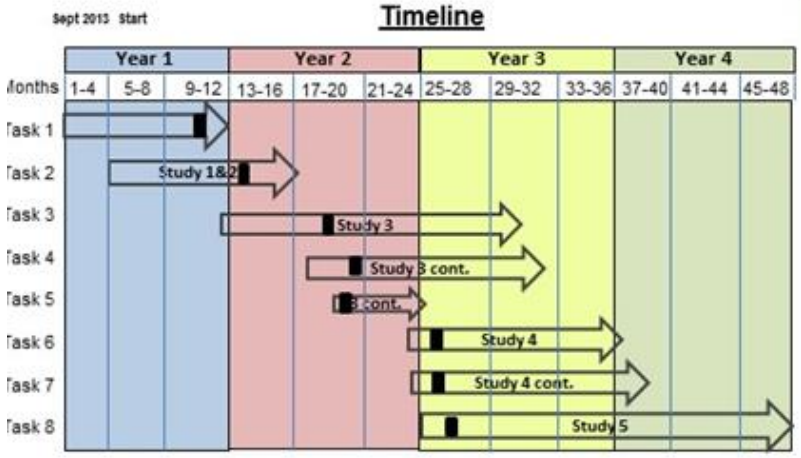
2-Morris group assessed cardiac function in mice model of GWI treated with DFP.

3-O' Callaghan group conducted dose response experiments for cyclosporine A, tacrolimus and Enbrel/Mifepristone Reset Therapy

4-Dr. Klimas received HRPO approval for HRPO approval for the Enbrel Mifepristone clinical trial.

5-Members of GWIC have submitted 8 GWI research grant proposals and 13 articles, 8 of which are under review.

Timeline



Goals/Milestones

FY13 Goal – Administrative structure for animal/human studies (Task 1)

- ☑ Kick-off meetings with GWIRP staff and study PIs
- ☑ Protocol preparation and initiation of approvals for animal/human use
- ☑ Coordinating center database set-up

FY14 Goal – Studies 1- 3 - Refinement and enhancement of models for GWI

- ☑ Establish model of autonomic dysfunction as a surrogate for GWI **(Task 2)**
- ☑ Identification of Illness specific networks with focus on human and mouse comparisons **(Task 3)**
- ☑ Large-scale simulation of treatment. **(Task 4)**
- ☑ Define/deploy optimization and target intervention possibilities **(Task 5)**

FY15 Goal – Study 4 - Candidate treatment courses

- ☑ Identify candidate treatment courses for GWI **(Task 6)**
- ☑ Select and test therapies in animals **(Task 7)**

FY16 Goal – Study 5 - Perform translational human clinical trials

- ☐ Verify treatment effectiveness in human subjects n=20 **(Task 8)**

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

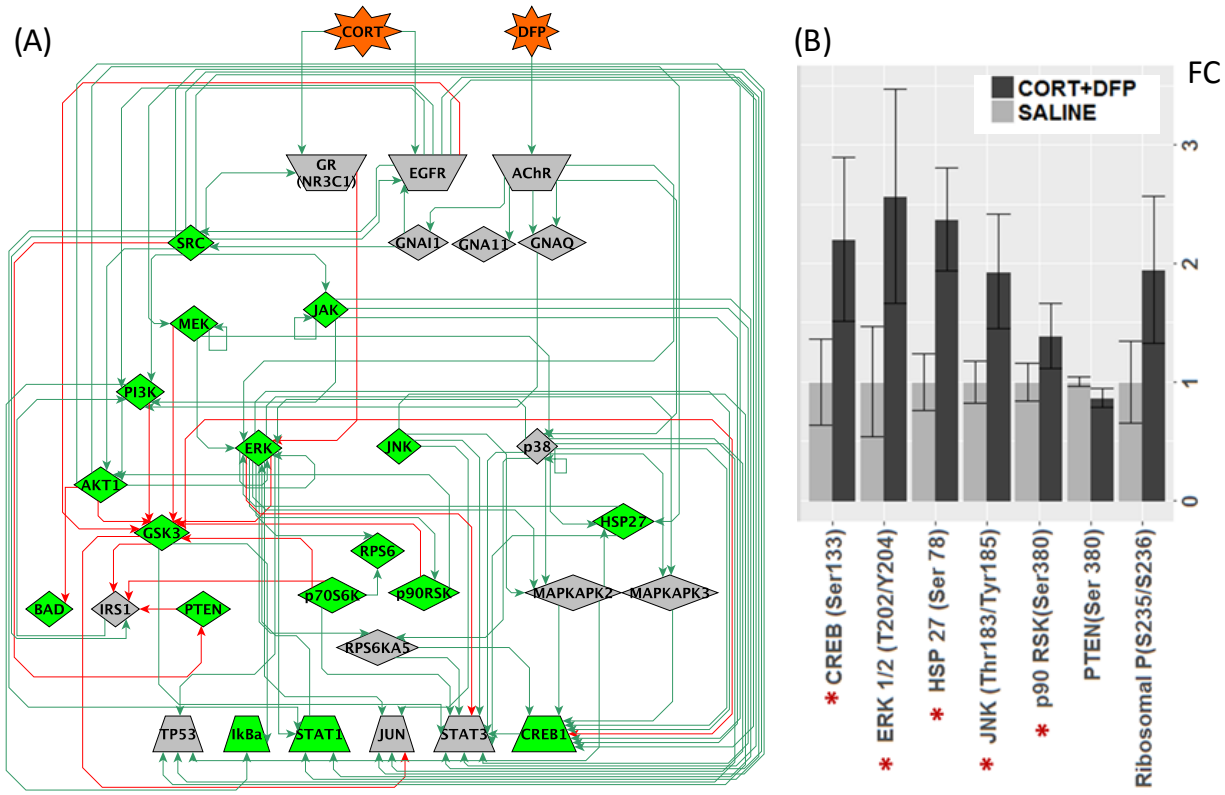


Figure 1. An Immune Signal Transduction Model of Stress Potentiated Response to DFP. A causal map of stimulatory and inhibitory molecular signals along a subset of core pathways involved in response to corticosterone (CORT) and the neurotoxin DFP (panel A). Interactions were identified by text mining the broad literature database available as part of the Pathway Studio suite (Elsevier Life Science Solutions, Rockville MD). Predictions from this partial preliminary model show broad alignment experimenatl data (panel B), agreeing with expression changes in 5 out of 7 proteins (red star *) measured during acute response to DFP exposure preceded by corticosterone administration. We expect tuning of the model logic to the prosed new data will further improve fidelity and predictive capability.

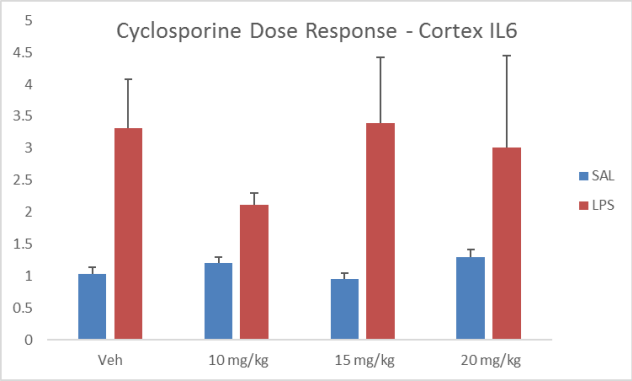


Figure 2. Dose response for cyclosporine A

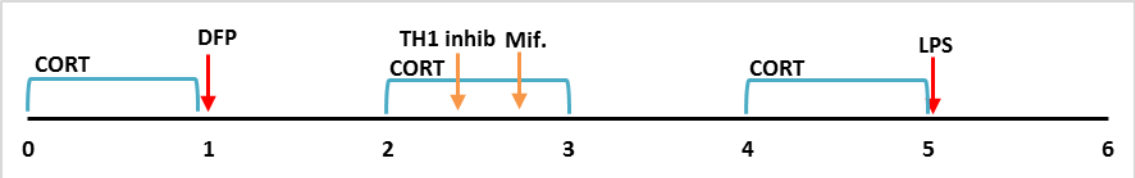


Figure 3. The Enbrel/Mifepristone Reset Therapy

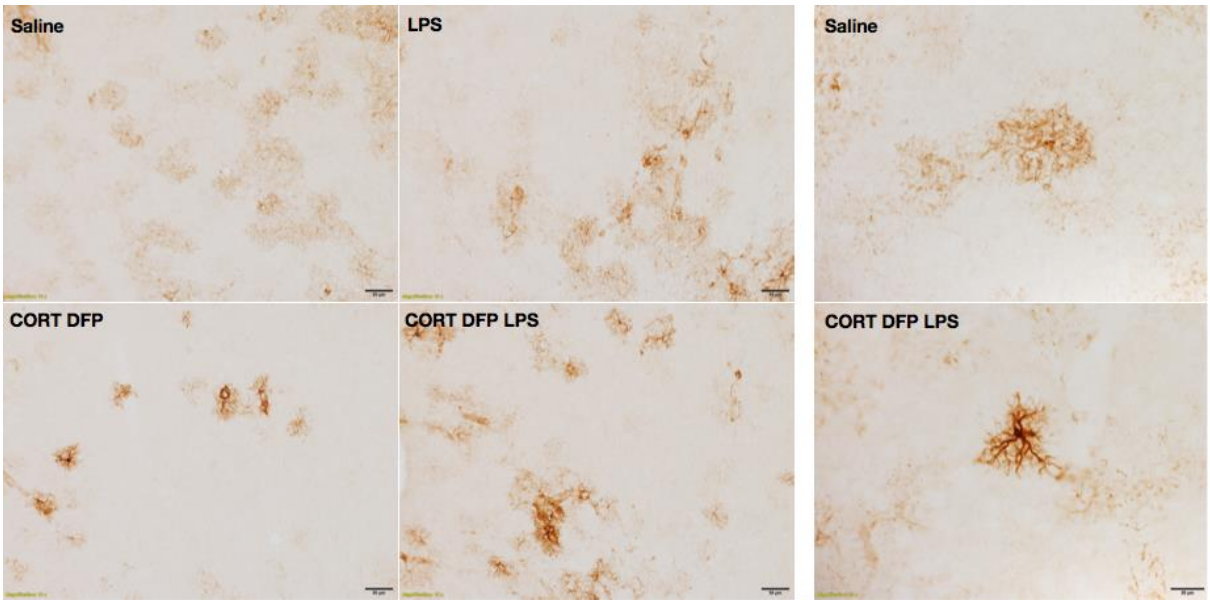


Figure 4. Astrocytes in the front Cortex appear to have increased hypertrophy (shown at 20x magnification)

Representative astrocytes (saline and CORT DFP LPS) are shown at 60x magnification in the panels to the right.

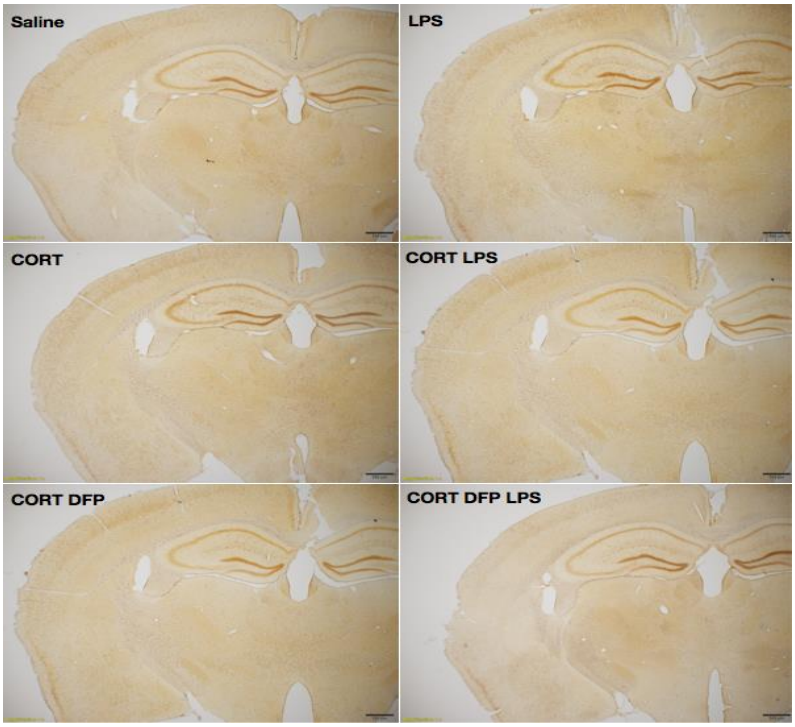


Figure 5. Silver stain for neurodegeneration 2x hippocampus

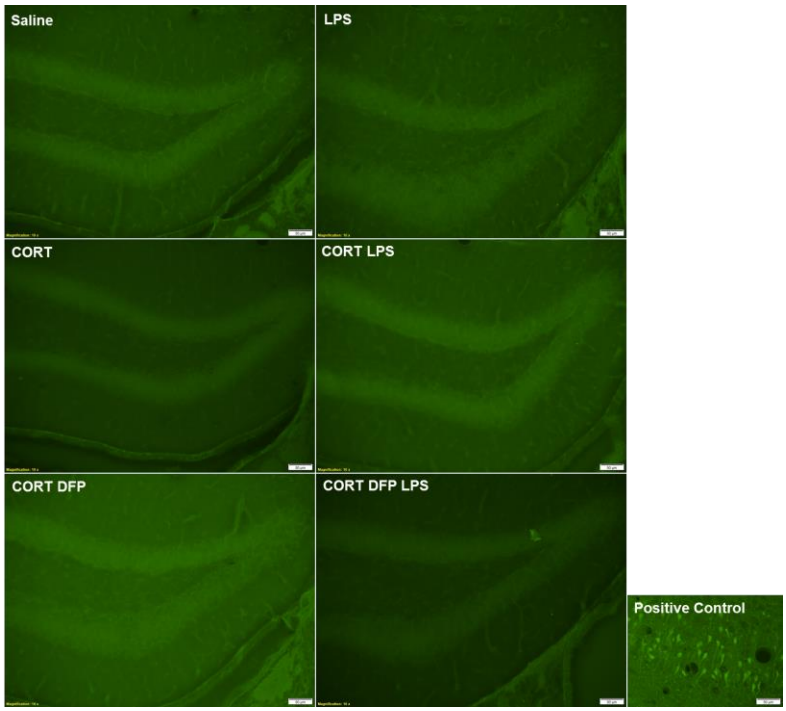


Figure 6. Fluoro-Jade B stain for neurodegeneration 20x magnification, hippocampus dentate gyrus shown. Positive Control image included at right.

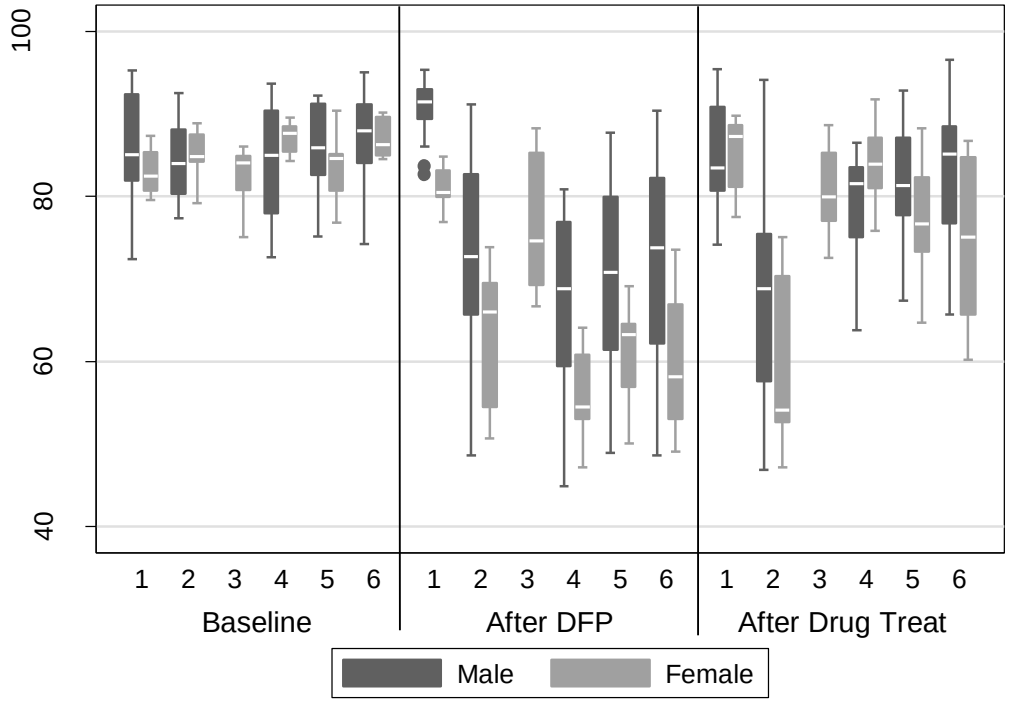


Figure 7A. Three time points male and female Ejection Fraction (%). Groups 1-6 please see legend.

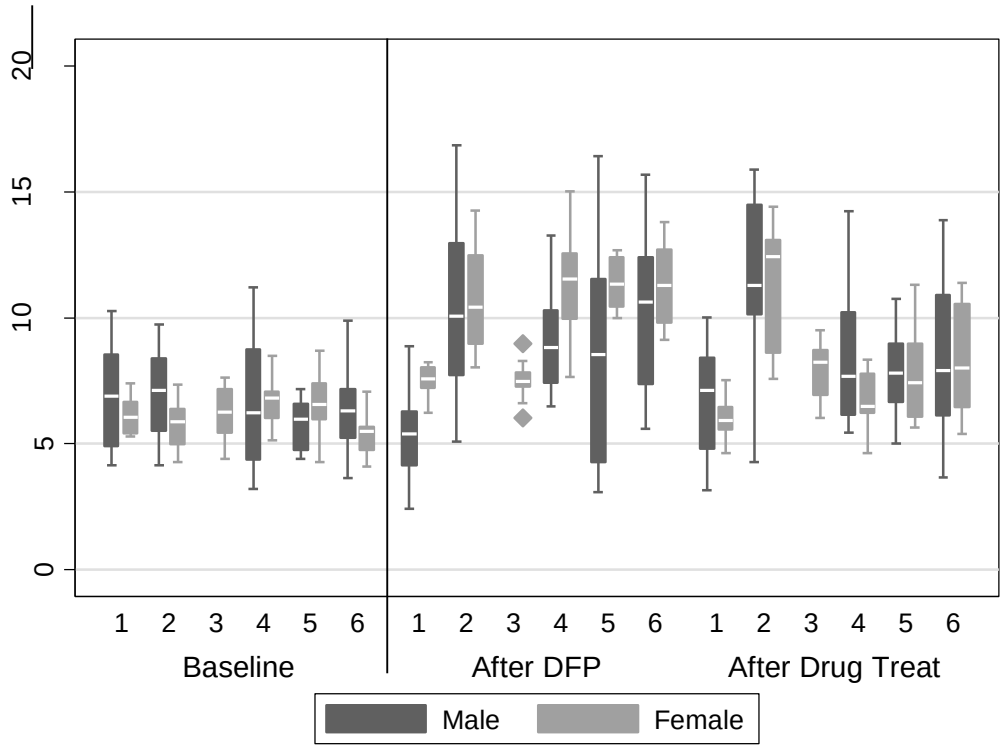


Figure 7B. Three time points male and female End Systolic Area (mm^2). Groups 1-6 please see legend.

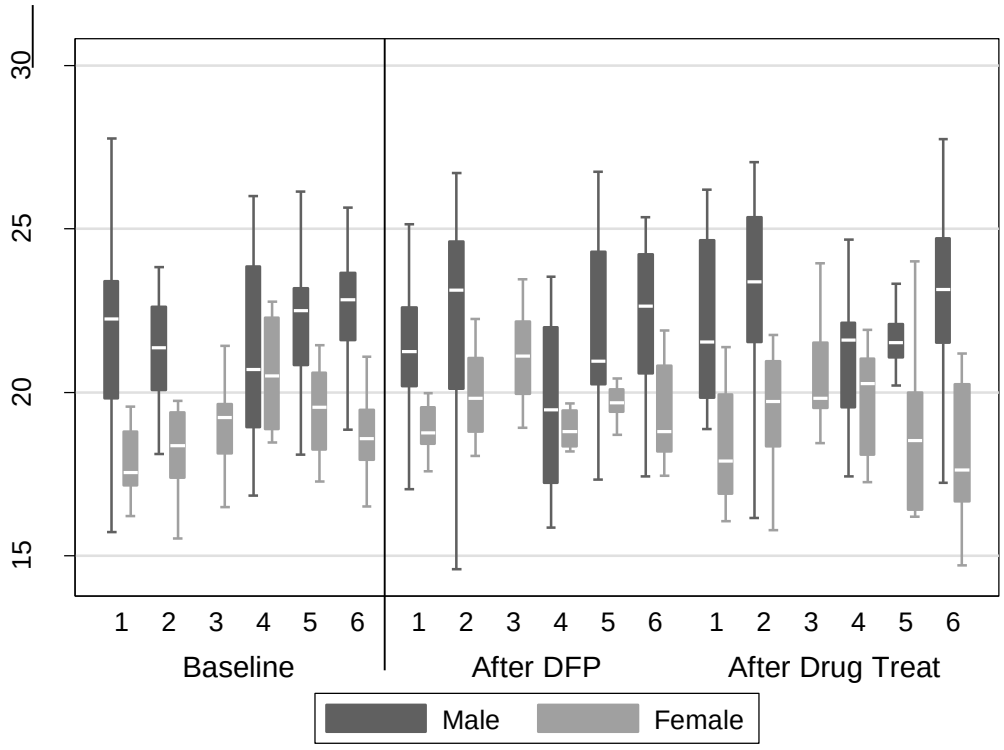


Figure 7C. Three time points male and female End Dyastolic Area (mm^2). Groups 1-6 please see legend.

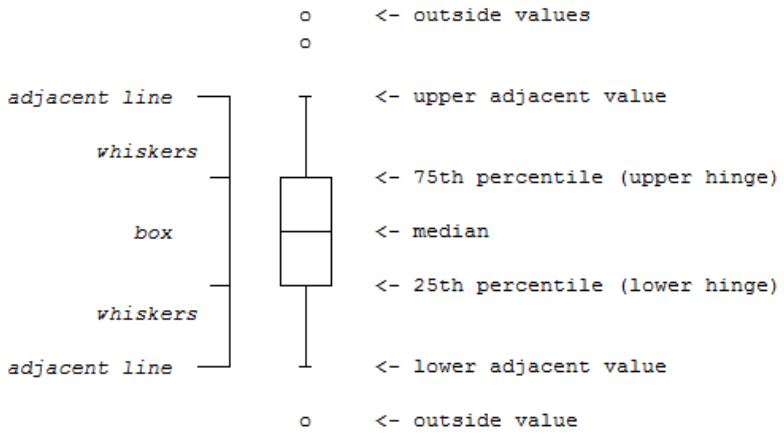


Figure 7D. Explanation of above box plots 7A-C.

Figure 8 C57BL/6J mice acquired from a commercial vendor will arrive to the USAMRICD. After the appropriate quarantine, the animals will be exposed to Sarin (0.4 LD50 injected subcutaneously on 2 consecutive days) or vehicle. Animals will be sent to the Miami VAHC-VMU after and appropriate recovery (up to 2-3 weeks). Upon arrival the animals will be allocated in five experimental groups. A series of cardiovascular evaluation (CV) will be performed at 6, 8, and +10 weeks post GB exposure. CV will include Transthoracic echocardiography (VeVo100), and non-invasive electrocardiogram (ECGenie), body density measures (Echo MRI). The 6-8 weeks protocols, can be extended (up to 12 weeks) to evaluate the persistent cardiovascular effect of the GWI exposure and/or the treatment of interest. Treatments can be planned at 14 days or any time after arrival to the VA. Neuro-immune and cardiovascular markers will be studied from tissue samples taken at the endpoint of the study.